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(21) International Application Number: PCT/US99/31098 (22) International Filing Date: 22 December 1999 (22.12.1999) (30) Priority Data: 60/113,730 23 December 1998 (23.12.1998) US (60) Parent Application or Grant NANOGEN, INC. [/]; O. CHENG, Jing [/]; O. WU, Lei [/]; O. HELLER, Michael [/]; O. SHELDON, Ed [/]; O. DIVER, Jonathan [/]; O. O'CONNELL, James, P. [/]; O. SMOLKO, Dan [/]; O. JALALI, Shila [/]; O. WILLOUGHBY, David [/]; O. CHENG, Jing [/]; O. WU, Lei [/]; O. HELLER, Michael [/]; O. SHELDON, Ed [/]; O. DIVER, Jonathan [/]; O. O'CONNELL, James, P. [/]; O. SMOLKO, Dan [/]; O. JALALI, Shila [/]; O. WILLOUGHBY, David [/]; O. MURDOCK, Douglas, C. ; O.		Published
(54) Title: INTEGRATED PORTABLE BIOLOGICAL DETECTION SYSTEM (54) Titre: SYSTEME DE DETECTION BIOLOGIQUE INTEGRE PORTATIF (57) Abstract We have performed separation of bacterial and cancer cells from peripheral human blood in microfabricated electronic chips by dielectrophoresis. The isolated cells were examined by staining the nuclei with fluorescent dye followed by laser induced fluorescence imaging. We have also released DNA and RNA from the isolated cells electronically and detected specific marker sequences by DNA amplification followed by electronic hybridization to immobilized capture probes. Efforts towards the construction of a "laboratory-on-a-chip" system are presented which involves the selection of DNA probes, dyes, reagents and prototyping of the fully integrated portable instrument. (57) Abrégé Nous avons procédé à la séparation de cellules bactériennes et cancéreuses de sang humain périphérique, dans des micropuces électroniques, par diélectrophorèse. Nous avons examiné les cellules isolées par coloration des noyaux avec un colorant fluorescent, puis par imagerie par fluorescence induite au laser. Nous avons également extrait de l'ADN et de l'ARN des cellules isolées, par voie électronique, et détecté des séquences marqueurs spécifiques, par amplification d'ADN, puis par hybridation électronique avec des sondes de capture immobilisées. Les efforts visant à l'élaboration d'un système de _ laboratoire sur puce _ sont présentés, notamment la sélection de sondes d'ADN, de colorants et de réactifs, ainsi que le prototypage de l'instrument totalement intégré portatif.		

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(54) Title: INTEGRATED PORTABLE BIOLOGICAL DETECTION SYSTEM

(57) Abstract

We have performed separation of bacterial and cancer cells from peripheral human blood in microfabricated electronic chips by dielectrophoresis. The isolated cells were examined by staining the nuclei with fluorescent dye followed by laser induced fluorescence imaging. We have also released DNA and RNA from the isolated cells electronically and detected specific marker sequences by DNA amplification followed by electronic hybridization to immobilized capture probes. Efforts towards the construction of a "laboratory-on-a-chip" system are presented which involves the selection of DNA probes, dyes, reagents and prototyping of the fully integrated portable instrument.

1. Sample Processing

Isolation from Crude Sample

Electronic Lysis

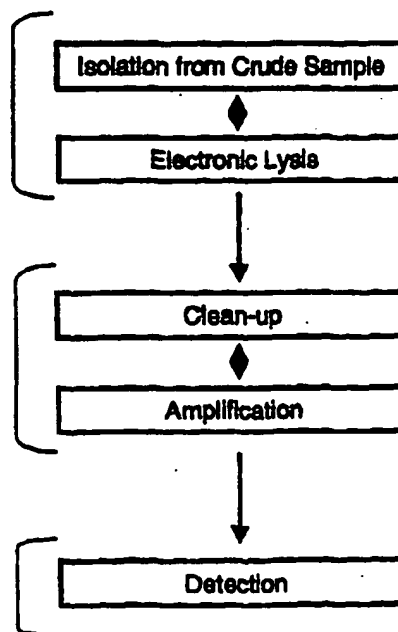
2. Chemical Reaction

Clean-up

Amplification

3. Analysis

Detection



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DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

INTEGRATED PORTABLE BIOLOGICAL DETECTION SYSTEM

GOVERNMENT SUPPORT

The Government has rights in this invention pursuant to Grant No. ATP:
70NANB7H3001 awarded by the Advanced Technology Program.

FIELD OF THE INVENTION

This invention relates to devices and methods for performing active, multi-step sample preparation and molecular diagnostic analysis of biological materials. More particularly, it relates to integrated, compact, portable devices for self-contained sample to answer systems. Specifically, this invention relates to a device and method for performing multi-step sample preparation and assay on either two or even a single microchip. Examples of applications for this integrated system include food and/or quality monitoring, diagnosis of infectious diseases and cancers, bone marrow plastesis (e.g., stem cell separation and analysis), and genetics-based identification of individuals for forensic purposes.

RELATED APPLICATIONS

This application claims benefit of provisional patent application no. 60/113,730, filed December 23, 1998, entitled "Fluorescent Imaging of Cells and Nucleic Acids in Bioelectronic Chips", and is a continuation-in-part of co-pending Application Serial No. 09/016,596, filed on January 30, 1998, entitled "Channel-Less Separation of Bioparticles on a Bioelectronic Chip by Dielectrophoresis", which is a continuation in part of co-pending Application Serial No. 08/709,358, filed on September 6, 1996, entitled "Apparatus and Methods for Active Biological Sample Preparation", and is a continuation-in-part of co-pending Application Serial No. 08/986,065, filed on December 5, 1997, entitled "Self-Addressable Self-Assembling Microelectronic Integrated Systems, Component Devices, Mechanisms, Methods, and Procedures for Molecular Biological Analysis and Diagnostics", which is a continuation-in-part of Application Serial No. 08/534,454, filed September 27, 1995, entitled "Apparatus and Methods for Active Programmable Matrix Devices", now issued as U.S. Patent No. 5,849,486, which is a continuation-in-part of Application Serial No. 08/304,657, filed September 9, 1994,

entitled "Automated Molecular Biological Diagnostic System", now issued as U.S. Patent No. 5,632,957, which is a continuation-in-part of Application Serial No. 08/271,882, filed July 7, 1994, entitled "Methods for Electronic Stringency Control for Molecular Biological Analysis and Diagnostics", now allowed, the specifications of which are hereby incorporated by reference as fully set forth herein.

BACKGROUND OF THE INVENTION

The following description provides a summary of information relevant to the present invention. It is not an admission that any of the information provided herein is prior art to the presently claimed invention, nor that any of the publications specifically or implicitly referenced are prior art to the invention.

Generally, analysis of biological-derived sample materials cannot occur until the sample is processed through numerous pre-analysis steps. Often, the preparation process is time consuming and laborious. For example, many immuno and molecular-biological diagnostic assays on clinical samples, such as blood or tissue cells, require separation of the molecules of interest from the crude sample by disrupting or lysing the cells to release such molecules including proteins and nucleic acids (i.e., DNA and RNA) of interest, followed by purification of such proteins and/or nucleic acids. Only after performing processing steps can analysis of the molecules of interest begin. Additionally, protocols used for the actual analysis of the samples require numerous more steps before useful data is obtained.

For example, charged and uncharged microparticles in solution (such as cellular material or crude extracts of protein or nucleic acids thereof) may be separated by dielectrophoresis. On a microscale, dielectrophoresis can be performed using a glass slide-based device having exposed, i.e., naked, interdigitated electrodes plated on the surface of the slide and having a flow chamber with a volume of several hundred microliters. With such a device, cells, proteins, and nucleic acids can be separated based on their respective dielectric properties by using separation buffers having appropriate conductivity and an AC signal with a suitable amplitude and frequency. Such devices, however, have several problems including the nonspecific binding of both separated and unseparated cells to exposed portions of the glass surface and the electrodes. Such devices are also problematic in that the flow chamber volume (several hundred microliters) is so large that thermal convection can disturb and push materials such as cells and large molecules initially attracted to and retained by the electrodes

off of the electrodes. Additionally, undesired cells and molecules are not easily washed off the surface without disturbing and loosing the desired cells as such cells and molecules can interfere with fluidic flow and, hence, block the flow during wash steps.

Conventional methods to disrupt whole cells for the release of proteins and nucleic acids have employed the use of a series of high voltage DC pulses in a macrodevice, as opposed to a microchip-based device. Such conventional electronic lysis techniques have several problems. For example, some commercial macro-devices use lysis conditions that do not release high molecular weight (larger than 20 Kb) nucleic acids because the high molecular weight molecules can not fit through pores created in the cell membranes using such methods. Additionally, released nucleic acids are often lost due to their non-specific binding to the surface of the lysis chamber. Such loss of material, especially when molecules of interest are in low concentration, is further compounded by the fact that the dielectrophoretic cell separation macro-device systems are stand alone systems allowing for loss of sample in the transfer of material from one device to the other as sample preparation is carried forward.

Processing of the crude lysate often requires chemical reactions to remove undesired cellular components from the specifically desired ones. These reactions typically include subjecting the lysate to enzymatic reactions such as proteinase K and restriction enzymes or nucleases. Processing can also include enhancing the presence of desired molecules, particularly nucleic acids, by performing amplification reactions such as by strand displacement amplification (SDA) or polymerase chain reaction (PCR) methodologies. These reactions are also carried out in stand-alone processes. Only after these sample preparation and processing steps can assaying for data retrieval begin. Because of the numerous steps between sample collection and assay, many a technique is limited in its application by a lack of sensitivity, specificity, or reproducibility.

Attempts have been made to use dielectrophoresis to separate and identify whole cells. For example, U.S. Patent No. 4,326,934 to Pohl discloses a method and apparatus for cell classification by continuous dielectrophoresis. With such method cells are separated by making use of both the positive and negative dielectrophoretic movement of cell particles. Separated cells are allowed to be characterized and/or classified by viewing the characteristic deflection distance of cells moving through the positive and negative electrodes.

5 In another example, U.S. Patent No. 5,344,535 to Belts et al. discloses a method and apparatus for the characterization of micro-organisms and other particles by dielectrophoresis. In this system, cells are characterized by matching their signature dielectrophoretic collection rates.

10 In yet another example, U.S. Patent No. 5,569,367 to Belts et al. discloses a method and apparatus for separating a mixture of cells using a pair of energized interdigitated electrodes comprised of interweaved grid-like structures arranged to obstruct flow of cells through the apparatus and cause differentiation of cell types into fractions by applying a non-uniform alternating field.

15 In addition, other attempts have been made to combine certain processing steps or substeps together. For example, various microrobotic systems have been proposed for preparing arrays of DNA probes on a support material. Beattie et al., disclose in "The 1992 San Diego Conference: Genetic Recognition", November, 1992, use of a microrobotic system to deposit micro-droplets containing specific DNA sequences into individual microfabricated sample wells on a glass substrate.

20 Various other attempts have been made to describe integrated systems formed on a single chip or substrate, wherein multiple steps of an overall sample preparation and diagnostic system would be included. A. Manz et al., in "Miniaturized Total Chemical Analysis System: A Novel Concept For Chemical Sensing", *Sensors And Actuators*, B1(1990), pp. 244-248, describe a 'total chemical analysis system' (TAS) that comprises a modular construction of a miniaturized TAS. In that system, sample transport, chemical reactions, chromatographic separations and detection were to be automatically carried out.

25 Yet another proposed integrated system by Stapleton, U.S. Patent No. 5,451,500, a system for automated detection of target nucleic acid sequences is described. In this system multiple biological samples are individually incorporated into matrices containing carriers in a two-dimensional format.

30 Various multiple electrode systems are also disclosed which purport to perform multiple aspects of biological sample preparation or analysis. Pace, U.S. Patent 4,908,112, entitled "Silicon Semiconductor Wafer for Analyzing Micronic Biological Samples" describes an analytical separation device in which a capillary-sized conduit is formed by a channel in a semiconductor device, wherein electrodes are positioned in the channel to activate motion of liquids through the conduit. Additionally, Soane et al., in U.S. Patent

5,126,022, entitled "Method and Device for Moving Molecules by the Application of a Plurality of Electrical Fields", describes a system by which materials are moved through trenches by application of electric potentials to electrodes in which selected components may be guided to various trenches filled with antigen-antibodies reactive with given charged particles being moved in the medium or moved into contact with complementary components, dyes, fluorescent tags, radiolabels, enzyme-specific tags or other types of chemicals for any number of purposes such as various transformations which are either physical or chemical in nature. Further, Clark, et al. in U.S. Patent 5,194,133, entitled "Sensor Devices", discloses a sensor device for the analysis of a sample fluid which includes a substrate having a surface in which is formed an elongate micro-machined channel containing a material, such as starch, agarose, alginate, carrageenan or polyacrylamide polymer gel, for causing separation of the sample fluid as the fluid passes along the channel. The biological material may comprise, for example, a binding protein, an antibody, a lectin, an enzyme, a sequence of enzymes, or a lipid.

Various devices for eluting DNA from various surfaces are known. For example, Shukla U.S. Patent 5,340,449, entitled "Apparatus for Electroelution" describes a system and method for the elution of macromolecules such as proteins and nucleic acids from solid phase matrix materials such as polyacrylamide, agarose and membranes such as PVDF in an electric field. Materials are eluted from the solid phase into a volume defined in part by molecular weight cut-off membranes. Also, Okano, et al. in U.S. Patent 5,434,049, entitled "Separation of Polynucleotides Using Supports Having a Plurality of Electrode-Containing Cells" discloses a method for detecting a plurality of target polynucleotides in a sample, the method including the step of applying a potential to individual chambers so as to serve as electrodes to elute captured target polynucleotides, the eluted material is then available for collection.

Other devices for performing nucleic acid diagnosis have been designed wherein at least two reaction chambers are necessary for carryout the sample preparation and analysis such as R. Lipshutz, et al., entitled "Integrated Nucleic Acid Diagnostic Device" (U.S. Patent No. 5,856,174) and R. Anderson, et al., entitled "Integrated Nucleic Acid Diagnostic Device", (U.S. Patent No. 5,922,591).

Still other achievements have been made toward partial integration of a complete sample handling system such as P. Wilding, et al., "Integrated cell isolation and PCR

analysis using silicon microfilter-chambers," *Anal. Biochem.*, **257**, pp. 95-100, 1998; and P.C.H. Li and D.J. Harrison, "Transport, manipulation, and reaction of biological cells on-chip using electrokinetic effects," *Anal. Chem.*, **69**, pp. 1564-1568, 1997.

Still others have attempted to integrate chemical reactions with detection such as M.A. Burns, et al., "An integrated nanoliter DNA analysis device," *Science*, **282**, pp. 484-487, 1998; S.C. Jacobson and J.M. Ramsey, "Integrated microdevice for DNA restriction fragment analysis," *Anal. Chem.*, **68**, pp. 720-723, 1996; L.C. Waters, et al., "Microchip device for cell lysis, multiplex PCR amplification, and electrophoretic sizing," *Anal. Chem.*, **70**, pp. 158-162, 1998; and A.T. Woolley, et al., "Functional integration of PCR amplification and capillary electrophoresis in a microfabricated DNA analysis device," *Anal. Chem.*, **68**, pp. 4081-4086, 1996.

Generally, as is understandable from the forgoing examples, systems and methods have been described that do not fully provide for a completely integrated self-contained sample to answer system that uses electronically active microchips. Moreover, numerous of the described systems are extremely labor and time intensive requiring multiple steps and human intervention either during the process or between processes which together are suboptimal allowing for loss of sample, contamination, and operator error. Further, the use of multiple processing steps using multiple machines or complicated robotic systems for performing the individual processes is often prohibitive except for the largest laboratories, both in terms of the expense and physical space requirements. For the reasons stated above, these techniques are limited and lacking. They are not easily combined to form a system that can carry out a complete self-contained integrated diagnostic assay, particularly assays for data retrieval for nucleic acids and protein-derived information, on a single electronically addressable microchip. Despite the long-recognized need for such an integrated system without a complicated fluidics and inadequate valve systems, no satisfactory solution has previously been proposed. There is therefore a continuing need for methods and devices which lead to improved dielectrophoretic separation of biological cells as well as improved biological stability of the separated cells and further a continuing need for methods and devices which improve cell preparation and analysis, and which are capable of integrating cell separation, preparation, purification, and analysis in a single self-contained system without complicated fluidics.

SUMMARY OF THE INVENTION

Accordingly, provided herein is an integrated, portable system and device for performing active, integrated multi-step sample preparation and molecular diagnostic analysis of biological samples using a minimal number of electronically addressable microchips.

In a preferred embodiment, the system and device has a single flow cell element for carrying out the separation, preparation, and analysis of preselected eukaryotic and/or prokaryotic cells from a mixed population of cells. This single flow cell element includes therein an electronically addressable microchip having a grid of individual electrodes. The grid can contain any number of individual electrodes and be fabricated on a silicon wafer as is well understood in the electronic arts. Generally, such a grid can comprise anywhere from 4 to 25 to 100 or even 10,000 or more individual electrodes. This single flow cell embodiment contemplates that the flow cell encompassing the electronic grid is able to achieve simple fluidic manipulation of the cells for their respective separation, lysis, clean-up of cell-derived materials of interest, and analysis of such materials. Preselected eukaryotic and prokaryotic cells may be any type (e.g., blood cells, cancer cells, stem cells, bacteria, etc.) and obtained from any source (e.g., animal, biopsy, blood, organs, forensic samples, stool, water, etc.). Specific examples of cells that can be separated from, for example, whole blood include *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus epidermus*, *Micrococcus lysodeikticus*, and cultured cervical carcinoma cells.

In an alternative embodiment, the device of the invention may include at least one additional flow chamber for carrying out either a variety of sample preparation steps or for analysis of prepared sample. For example, in one alternative embodiment, the system comprises a first and second flow cell chambers each having an electronically addressable microchip. In this alternative embodiment, the first chamber may be used to separate and lyse cells as well as perform sample preparation steps, while the second chamber is used to analyze the sample materials directly on the second flow cell microchip.

In another alternative embodiment comprising at least two flow cells, the first flow cell has no electronically addressable grid but does have at least a heating element attached thereto that can be used to adjust the temperature of materials within said cell. The second flow cell is contemplated to have an electronically addressable microarray for

5 use in performing analysis of the sample materials.

10 Regardless of the embodiment utilized, the system of the invention provides the ability to (1) separate eukaryotic cell types from one another as well as eukaryotic cell
15 types from prokaryotic cell types, (2) directly process the sample materials from a crude state to a more refined state, and (3) directly analyze the sample materials on the
20 microchip grid. Such an ability is possible by the novel use of electronic biasing at one level of voltage in the form of a dielectric current to cause dielectrophoresis of cells,
25 followed by an increase in voltage to lyse captured cells, followed in turn by changing the manner of biasing from an alternating current mode to direct current mode for the
30 addressing of specific electrodes on the arrays of the flow cell(s) to cause the transport of molecules of interest for capture/hybridization to probes previously bound to the electrode
35 array. The system of the invention further contemplates that other appropriate sample preparation reagents may be transported to and away from the flow cell(s) by simplified arrangement of tubing and solenoid operated valves and pump. Additionally, in
40 15 embodiments having a first flow chamber that is without a microchip, the system contemplates the ability to directly lyse the cells in the sample and analyze materials of interest without a need for separating the cell types. In such embodiment, the flow cell has
45 a heating element that can be used to raise the temperature for direct lysis of the cells in the sample. Following such lysis, sample preparation steps such as protease treatment or
50 20 nucleic acid amplification may be performed followed by transporting the amplified species to the second flow cell containing the electronically addressable microchip.

35 In another embodiment, the integrated system contemplates the direct processing of cells selectively separated or lysed in the first chamber to obtain any materials of interest from such cells. Such processing is generally directed to the isolation and
40 25 purification of proteins and nucleic acids from cellular contaminants. Numerous techniques can be performed in the preparation of molecules of interest including, but not limited to, enzymatic treatment using protease K to remove proteinaceous materials from
45 nucleic acids of interest, enzymatic treatment using nucleases to remove nucleic acids from proteins, digestive residue adsorption, nucleic acid amplification (e.g., by PCR and
50 30 SDA), *in situ* buffer exchange and binding of antibodies or other protein-protein binding reactions such as receptor-ligand or enzyme-substrate for binding to proteins of interest.

55 In another embodiment, analysis of prepared sample materials can comprise any

number of preselected hybridization formats. In a preferred format, nucleic acids of interest are hybridized selectively through an electronically directed process as is known to those skilled in the art of electronically addressable microchips. Such hybridization formats comprise binding of nucleic acids (RNA, DNA, pNA) to probes anchored to the microarray. Other formats contemplated for use with the system include selective capture of proteins of interest such as by antibody or other protein binding probes attached to the electronic grid. These can include other protein-protein interactions such as receptor-ligand and enzyme-substrate binding.

In still other embodiments, the device contemplates elements (e.g. buffer vials, tubing, miniature solenoid valves, and at least one pump) for carrying and transporting samples, buffers, enzymes and reagents to and from said flow cell(s). Additionally, yet other embodiments are provided for detecting the analytes and molecules of interest being assayed. In a preferred embodiment, such elements include a battery operated diode laser (preferably having a wavelength of 635 nm), and a CCD camera coupled with filters and zoom lens for astronomy of the individual electrodes of the microchip grid(s) of the first and/or second flow cell chamber(s). Still other methods of detection are also contemplated not requiring illumination with a light emitting device such as direct electrochemical detection as is well known by those in the art of such detection as described in P. Ropp and H. Holden Thorp, *Chemistry & Biology*, 1999, Vol. 6, No.9, pp. 599-605. Each of these electronic components are further contemplated to be coordinated through a computer and appropriate programming software as is well understood by those skilled in the electronic arts.

BRIEF DESCRIPTION OF THE DRAWINGS

The file of this patent contains at least one drawing executed in color. Copies of this patent with color drawing(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee. The invention will be further described with reference to the accompanying drawings in which:

Figure 1 is a simplified flow chart of the system showing three basic stages of the system including sample preparation, chemical reaction and analyte detection.

Figure 2 is a schematic showing the simple overall hardware design of the system wherein there is included a flow chamber 11 with heating element 12, a miniature

desalting column 13, a laser 14 with optical path 15, and a detector such as a CCD camera 17.

Figure 3 is a perspective view showing one example of an external design for the portable system of this invention. The portable system comprises a housing 18 integrated with a computer and monitor 20 via a cable 19.

Figure 4 is an internal view of the integrated system showing various components thereof such as sample and reagent vials 21a-e, heating elements 12, CCD camera 17, laser 14, a series of pumps 46 for each reagent vial, and other features such as a focus adjustment knob 4 and area for electronic components 5.

Figure 5 is a cross-section diagram for a flow cell having a microchip grid. The flow cell in total comprises a microchip grid 23 on the inner bottom surface of the flow cell chamber 29. The flow cell is covered by a quartz window 28. The underside of the microchip substrate has attached thereto a heating element 12. The flow cell has at least two inlet ports 30.

Figure 6 is a schematic showing an alternative embodiment having two flow cells. In this embodiment, a first flow cell can comprise either a cell having an electronically addressable microchip 31a, or not having such a microchip 31b. In either case, flow cell 31a,b has attached thereto a heating element 12. In this embodiment, the second flow cell 32 has an electronically addressable microchip attached to a heating element 12. As with the preferred embodiment diagramed in Fig. 2, the integrated system further includes a desalting column 13, laser 14 with light path 15, and a detector 17 positioned on a movable track 33 for positioning the detector for astronomy of either flow cell.

Figure 7 is a schematic showing a two flow chamber embodiment wherein first 31a and second 32 flow cells are arranged adjacent to one another. Also depicted are flow systems for channeling buffers, reagents, and analytes through the flow cells and desalting column of the system.

Figure 8 shows bias pattern formats that can be used to create a pattern of dielectric force for separation of eukaryotic or prokaryotic cells. In (A) is depicted a square-wall dielectric force pattern. In (B) is depicted a checkerboard force pattern.

Figures 9 and 10 show two computer representations of dielectric field distributions corresponding to either a square-wall or a checkerboard force pattern respectively.

Figure 11 shows a photograph of an electronically addressable microarray in a flow cell during cell separation. The photo specifically depicts a bacterial species being separated from whole blood using a checkerboard dielectric force pattern applied to the electrode of the flow chamber. The bacteria are the light colored concentrations at the field charge maximums directly above the electrodes of the array while the blood cells are in between the electrodes at the field charge minimum areas.

Figures 12, 13A and 13B show a eukaryotic cells (cultured HeLa cells) being separated from blood cells (Fig. 12) and after washing (Fig. 13A) and staining with propidium iodide (Fig. 13B). The mixture of the normal human white and red blood cells was pushed to the space between the electrodes where the field was minimum as reflected by the pinkish diamond-shaped spot (Fig. 12).

Figures 14-17 show a series of photographs where bacteria cells are separated from blood cells using either a square-wall or checkerboard dielectric field pattern. Figs. 14 and 15 show such separation for square-wall and checkerboard patterns respectively. Figs. 16 and 17 show separated bacteria after washing square-wall and checkerboard patterns respectively.

Figure 18 is a photo of a PAGE showing analysis of nucleic acids released from bacteria by electronic lysis. Lanes 1 and 6 are markers, lanes 4 and 5 are whole bacteria DNA with and without Rnase treatment respectively, and lanes 2 and 3 are supercoiled and linear plasmid pCR2.1 DNA released from the bacteria.

Figures 19 and 20 are PAGE photos showing amplification of two *S. enterica* genes *spa Q* and *inv A*, by SDA in the flow cell of the invention. In Fig. 19, from left to right, lanes 1 and 6 are size markers, lanes 2 and 3 are negative and positive controls respectively, lane 5 is the result of an amplification of *inv A* gene specific DNA sequence amplified using the device of the invention. In Fig. 20 lane 2 is a marker, lanes 1 and 4 are positive and negative controls respectively, and lane 3 is the amplification result for the *spa Q* gene.

Figure 21 is a photo showing hybridization results for *spa Q* gene wherein two electrode pads equipped with specific probe for *spa Q* gene were tested for hybridization in duplicate, a third pad specific for the gene was hybridized with amplified material from a positive tube reaction, and a fourth pad which was equipped with a nonspecific probe was tested for hybridization to amplification product produced in the flow cell.

Figure 22 is a photo showing amplified *spa Q* gene products addressed to capture sites of electrodes of the microarray using two different biasing conditions. In the top row is duplicate addressing to pads having capture probes specific for *spa Q* sequence (lanes 3 and 4) while lanes 1 and 5 have nonspecific probes. In the top row, a sinusoidal wave AC format was used while in the bottom row a direct current (DC) format was used. The results indicate that using a DC format is slightly superior to an AC format in transporting nucleic acids to the electrodes as the DC protocol bound a higher degree of amplicon.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Referring now to specific embodiments of the invention, a portable lab-on-a-chip system is provided wherein a sample may be processed and analyzed in at least a single flow cell comprising an electronically addressable microchip. The elements of the device are housed in a portable casing or housing which contains the sample preparation and analysis flow chamber, fluid handling systems, and illumination source (such as a battery operated 635 nm diode laser) and detection electronics (such as a CCD camera coupled with a set of filters and a zoom lens for astronomy of the microchip). External to the portable casing is a computer, such as a personal computer connected to the portable housing by a cable.

Referring now to Fig. 1, a flow chart is shown depicting three distinct sample handling stages for carrying out bioassays as contemplated using the lab-on-a-chip system of the invention. These are: (1) sample processing, (2) chemical reaction, and (3) analyte detection.

Sample handling in stage (1) processing generally comprises processing of crude biological samples (e.g., cells from blood, urine, stool, mixed cell populations, etc.) for the purpose of isolating molecules of interest such as nucleic acids and proteins. Sample handling at the chemical reaction stage (2) involves potentially many types of molecular biological reactions for clean-up and further isolation, purification, or amplification of molecules of interest including, but not limited to, enzymatic-based reactions such a treatment with proteases, nucleases and restriction enzyme digestion, PCR and SDA-based nucleic acid amplification, *in situ* buffer exchanges, chemical labeling such as by radioisotope and fluorescence markers, and immuno-based and protein-protein reactions such as antibody-antigen, ligand-receptor, and enzyme-substrate reactions. Sample

5 handling at the analyte detection stage (3) may be accomplished through numerous
formats including optical detection of fluorescent emissions, electrochemical detection,
10 and radioisotope detection. In a preferred embodiment, this detection comprises
hybridization of nucleic acids or captured proteins to the electronic grid and detection
5 using fluorescent imaging.

In Figure 2, the system is depicted in schematic form showing a top-down or
15 edgewise view of the basic components. In this diagram a support 10 is used to mount the
flow cell 11. On the backside of the flow cell 11 mounted to the support is a heating
element 12. Additionally, the flow cell may be connected via a hollow fiber to ports and
20 to a desalting column 13 in order to purify, desalt and introduce different buffers into the
flow stream. The heating element may be used to heat the flow cell for either direct heat-
induced lysis of cells in the chamber or for use in stage 2 process reactions such as
inhibiting enzymes and temperature cycling for nucleic acid amplification.

As will be understood by one of skill in the art, the flow cell, as well as the other
25 electrically operated components, such as solenoid operated valves and pumps, laser and
detection camera, are interconnected electronically with the computer 20 by cabling 19 to
the housing 18 for all programming and control purposes. By way of example, the power
source and computer are connected to the electrodes of the flow cell 11 for programming
30 and manipulation. In the preferred embodiment, the electronic signals that may be
generated by the computer software used with the system include an AC component, such
as where the AC component is sinusoidal and used in dielectrophoresis, and such as where
a DC component is square and used in hybridization. In yet a further aspect, it is preferred
35 that a time varying electronic signal also used in the system includes an offset signal, such
as where the offset signal is a DC signal.

40 Figure 2 further shows illumination source 14 that emits a laser beam 15.
Preferably, the illumination source 14 is a diode laser. The beam 15 is preferably incident
on a beam splitter 16 that directs at least a portion of the beam 15 towards the flow cell 11
microchip for sample analysis. Radiation emitted from the microchip grid electrodes
45 retraces the incident path, and at least a portion of that radiation is passed through the
beam splitter 16 to detector 17. In the preferred embodiment, the detector 17 comprises a
charged coupled device (CCD) detector. Alternatively, other detectors may be utilized.
50

5 However, for compactness, it is preferred to have a detector which generally has relatively larger area coverage compared to its depth (in the direction of the emitted radiation).

10 Figure 3 shows a perspective view of one design or style for the overall portable, integrated lab-on-a-chip system. A computer 20, preferably a portable notebook personal
5 computer, includes conventional elements such as a keyboard, function keys, a monitor, and input devices, e.g., trackball, and mouse. Housing 18 serves to contain not only the preparation, reaction, and analysis platforms comprising the flow cell, but also additional
15 equipment utilized in conjunction with computer 20 for operation of the system. For example, housing 18 may contain (Fig. 4) a power supply, waveform generator, laser, flow cell(s), CCD camera, and other electronics, fluidic systems, and reagents required for
20 operation and control of the system.

As an example of a flow cell design, Figure 5 shows a cross-section of such a cell. An electronically addressable microarray 23 is mounted onto substrate 10 containing a 1.0
25 inch square pin grid array (PGA) (068 PPGA, 400 Square Cavity, Spectrum Semiconductor Materials, San Jose, CA). Attached to the back side of the substrate 10 is a ceramic chip heater element 12 (Dawn 505, Dawn Electronics, Carson City, Nevada). The heating element 12 may be attached by any number of methods. In a preferred
30 embodiment, the attachment may be achieved by placing a thermally conductive flexible adhesive 22 between the backside of the electronic microchip and the ceramic heater and then incubating at an appropriate temperature and time to fix the adhesive. Additionally,
35 as is well understood in the art, these microchips are coated with a thin permeation layer 25 such as a hydrogel, agarose, a polymer of acrylamide, or a sol-gel matrix or the like. This permeation layer 25 protects the cells and molecules of interest (i.e., biomaterials) from the electrochemistry occurring at the electrode surface that would otherwise damage
40 the biomaterials (or the ability to assay them) if they were exposed directly to the electrodes.

The microchip can be then attached to a molded or machined medical grade plastic flow cell 27 so that the electronic microarray 23 makes up the inner bottom surface of a
45 well within the flow chamber. The flow cell 27 provides a compartment 29 for containing biological sample materials and buffers to be layered on top of the microchip. The flow cell may be further designed to have at least 2 to 4 ports 30 for sample delivery and
50 extraction. The flow cell 27 may further be constructed so as to accept tubing for

5 interfacing with exterior fluidic delivery and removal systems as well as desalting. Additionally, the flow cell compartment is covered and sealed by a fused quartz window or lid 28 for visual access to the microarray analysis sites or capture pads. This window
10 28 can comprise any thickness of quartz but generally is about 0.015 inches thick.

5 The window 28 may be attached to the flow cell and the flow cell to the chip by any number of methods of which a preferred method uses an ultraviolet (UV) curing adhesive 26 developed for optics assembly. Attachment of the flow cell to the chip may
15 be either directly to a bare surface of the chip or to the permeation layer overlaying the microarray also using adhesive 26. Tubing is attached to the inlet and outlet ports using a variety of methods depending on the tubing size, fittings, and the tubing base material.
20

In an alternative embodiment, the integrated system may use two flow cells, one for sample preparation and the second for analysis. Figure 6 shows in schematic form such an embodiment. The first flow chamber 31 is attached to a heating element 12 and may optionally have an electronically addressable microchip 31a or no such microchip
25 31b. In either format, i.e., with or without electronic microchip, the flow cell 31 is used to perform in a stepwise fashion cell separation and/or cell lysis, and clean-up and further preparation of molecules of interest recovered from the lysed cells. Further preparation of molecules of interest is contemplated to include any number of chemical reactions and steps as stated above such as isolating specific proteins of interest, isolating nucleic acids
30 of interest such as by treatment with proteases to remove proteinaceous material from the nucleic acids, and amplification of nucleic acids of interest such as by PCR and SDA.

35 The second flow cell 32 is used for sample analysis. In a preferred embodiment both flow cells 31 and 32 are disposed on the same side of the support 10. However, these flow cells may be disposed in alternative arrangements, including a back-to-back or a
40 25 stacked configuration. Additionally, these two flow cells may be connected via a hollow fiber in order to purify, desalt and introduce different buffers into the flow stream. Moreover, each flow cell may be designed to have independent temperature control elements as desired. As will be understood by one of skill in the art, each of the electronically addressable microchips within the flow cells, as well as the other electrically
45 30 operated components, such as solenoid operated valves and pumps, are interconnected electronically with the computer 20 by cabling for all programming and control purposes as in the single flow cell arrangement.

Figure 6 further shows illumination source 14 that emits a laser beam 15 and operates in the same fashion as described above. A camera with zoom lens 17 for astronomy of the electronic grids of the flow cells is also incorporated on a movable track 33 so that both the first and second flow cell electronic microarray grids can be viewed.

Figure 7 is a schematic of the alternate system showing support 10 including the flow cells for sample preparation 31a (shown as an electrode comprising flow cell) and analysis 32 (shown as a 25 electrode grid). Each of the flow cells preferably include a plurality of ports 42 connected to tubing or hollow fiber 44 leading to electrically operated 3-way valves 45. With respect to the preparation flow cell 31a, the cell is contemplated to possess at least two ports 42, optionally at least three ports, and in the preferred embodiment four ports. With respect to the analysis flow cell 32, this cell preferably has two ports. These ports 42 and their respective tubing 44 may be utilized for various functions at different times. For example, at one time a port may be considered an input port, whereas at another time, it may comprise an output port, based on the directionality of the flow into or out of the flow cells 31 and 32 as directed by the setting of the 3-way valves 45 and directionality of pump 46. Fluids in the chambers may be mixed by "pushing" and "pulling" of the liquids in various sequences through the ports. Such mixing may be advantageous, for example, during an amplification procedure.

Figure 7 further depicts other components of the self-contained system. In one embodiment, receptacles 21a-e provide for carrying samples and reagents such as for washing, amplification, hybridization, and waste. In another embodiment, the support 10 has attached thereto a miniature desalting column or a hollow fiber ion-exchange unit 13 for desalting small sample volumes. Often, there is a need to perform a desalting step to lower the ionic strength of the sample prior to attempting to electronically address amplified nucleic acids to specific capture pads of the microarray. This is because reagents used in amplification steps have an ionic strength that interferes with the migration of molecules of interest under the electronic addressing protocols. Once the amplified sample has been desalted it can be directed to the flow cell 32 for analysis on the microarray.

Whether using a single or multiple flow cell integrated system of the invention, cell separation is achieved by a dielectrophoresis technique. In such method, polarizable

5 particles (e.g., eukaryotic and prokaryotic cells) including those with no net charge are
subject to a "dielectrophoretic" force of a non-uniform electrical field. As long as the
10 effective polarizability of the particles is different from that of the surrounding medium,
the cells will remain subject to the dielectric force. The direction of migration of different
5 cell types is determined by: (1) surface charges of the cell walls or membranes of the cell
bilipid membranes, (2) the conductivity and permittivity of such cell membranes and walls,
15 and (3) the morphologies and structural architectures of the cells. Dielectrophoresis as
practiced in the current invention has been used to selectively separate from mixed cell
populations (e.g., blood cells) several types of bacteria including *Escherichia coli*,
10 *Salmonella typhimurium*, *Micrococcus lysodeikticus* and *Staphylococcus epidermidis* as
well as cancer cells such as cultured cervical carcinoma cells.

In a preferred embodiment, a flow cell useful for separating cells as practiced in
this invention may be designed to include an electronically addressable microchip having
25 at least 100 individually addressable microelectrodes capable of being biased individually
15 so that a dielectric force pattern may be generated across the microchip electronic grid.
For example, Fig. 8 shows two such force patterns. In Fig. 8A is a square-wall pattern
while 8B shows a checkerboard pattern. Each of these patterns provide a unique strong
30 and weak ionic field strength pattern that provides dielectric force sufficient to separate
different cell types. The force pattern is further depicted in computer generated field
20 patterns shown in Figs. 9 and 10 for square-wall and checkerboard respectively.

35 Additionally, the electrodes of the microarray in the flow cell(s) may be biased
either all together as a single array for carrying out cell separation, lysis, and analysis, or
may be programmed to form subarrays in any number of patterns for carrying out the
separation, lysis, and analysis steps. In other words, one subset may be biased to separate
40 25 cells, another set may be biased to cause cell lysis, and yet another set may be used for
analysis.

In an example of cell separation, Figure 11 shows the separation of *Micrococcus*
45 *lysodeikticus* from whole blood. The bacteria are concentrated above the electrodes while
the undesired blood cells, both red and white cells, are dark areas in between the
30 electrodes. The cells were made to separate using a protocol of biasing comprising a
sinusoidal signal of 10 V peak-to-peak at 10 kHz. Although this signal value was used in
50

5 this instance, generally cell separation can be carried out using sinusoidal signals wherein the Volts are between 2 and 20 peak-to-peak while frequencies are between 5 and 50 kHz.

10 Undesired cells are washed from the flow chamber while retaining the cells of interest. This is accomplished by maintaining an attractive bias for the cells of interest
5 and creating a flow of wash buffer through the flow chamber. Once the cells of interest are isolated in the flow chamber they may be treated in any number of ways for further processing. In one embodiment, the cells are lysed by applying high voltage pulses of up
15 to 450 volts with a pulse width between 10 μ s and 50 μ s as described in J. Cheng, et al., "Preparation and hybridization analysis of DNA/RNA from *E. coli* on microfabricated
10 bioelectronic chips," *Nature Biotechnol.*, 16, pp. 541-546, 1998. In another example, *E. coli* cells were separated from blood cells as shown in Figures 14-17. In Figure 14 a square-wall dielectric force pattern was used while in Fig. 15, a checkerboard pattern was used. As an example, the separation for the checkerboard pattern used a bias format of a
20 sinusoidal signal of 10 V peak-to-peak at 10 kHz. Figures 16 and 17 show how cleanly the bacterial cells were isolated following washing of the square-wall and checkerboard
25 patterned separation.

30 In another example, mammalian cells are separated on the electronic grid of the flow chamber. Figures 12, 13A and 13B show separation of cervical carcinoma cells. Fig. 12 shows initial separation wherein the cells move onto the electrodes of the grid
20 where the field is at a maximum by subjecting the cells to a positive dielectrophoretic force. The mixture of the normal human white and red blood cells mixed with the carcinoma cells are pushed to the space between the electrodes where the field was minimum as reflected by the pinkish diamond-shaped spots. After washing, the carcinoma cells were retained by the electrodes (Fig. 13A) whereas all of the normal blood
35 cells were washed out. Figure 13B shows the cells after staining with propidium iodide. As an example, the mammalian cells could be separated using the checkerboard format and applying a sinusoidal signal of 6 V peak-to-peak at 30 kHz for 3 minutes. J. Cheng, et al., "Isolation of Cultured Cervical Carcinoma Cells Mixed With Peripheral Blood Cells
40 on a Bioelectronic Chip," *Anal. Chem.* v.70, pp2321-26, 1998.

45
30 Following lysis, both cellular proteins and nucleic acids may be retained for analysis. An example showing that nucleic acid may be retained from lysed cells is
50
55

provided in Figure 18. There, *E. coli* DNA is run on a PAGE gel showing both chromosomal and plasmid DNA is retained.

Where nucleic acids are desired for analysis, further clean-up and purification may include treatment with proteases such as with Proteinase K. After such treatment, the lysate may either be analysed immediately, or amplified then analysed. If the alternate embodiment is used, the sample may be further treated in the flow chamber or may be exported to the second chamber for such protease treatment, amplification and analysis. Generally, a protease treatment may be carried out in an appropriate buffer at 60°C for 15 min. Temperature control may be achieved by use of the ceramic heater element attached to the back of the microchip. Inactivation of proteinase K may be achieved by heating the sample at 95°C for 2 min. Overall, the cell separation, lysis, and protease treatment process can take between 15-25 minutes.

Where proteins are desired, nucleic acids may be removed by treating the sample with restriction enzymes and nucleases. The proteins may further be treated with various enzymes and partial protease treatments to release specific proteins of interest from cell membranes and other cellular components. Following the above sample processing steps additional processing may be carried out including amplification (e.g., by PCR and SDA) and labeling with radioisotopic or fluorescent markers.

In an example of chemical reaction stage processing, specific nucleic acid sequences, (i.e., the *invA* and *spa Q* genes of *Salmonella enterica*) were amplified using SDA. SDA is preferred in this portable system as the amplification may be performed under isothermal conditions at between 50°C and 60°C thereby eliminating high temperature denaturation cycles associated with PCR. However, in one embodiment, the portable system is capable of performing PCR amplification as the flow chamber is equipped with the aforementioned heating element that has the capability of achieving repetitive high temperature cycling.

As shown in Figures 19 and 20 SDA can be effectively performed in the device with a product yield comparable to the positive control performed in a conventional reaction tube. In this example in a volume of 50 μ l, *Salmonella enterica* and blood cells were mixed and injected into the flow cell containing a 10,000 site electrode array. The bacterial cells were separated from the blood cells by dielectrophoresis using a biasing

5 protocol of a 10V peak-to-peak signal having a sinusoidal wave form with frequency of
between 10 and 15 kHz using the checkerboard pattern. The cell mixture was pumped
10 into the flow cell at a rate of 31 μ l per minute until the flow chamber was filled, then the
flow rate was adjusted to 12.4 μ l/min until the total 50 μ l volume was passed through the
5 chamber. The cells were retained above the electrodes by influence of the positive
dielectrophoretic force established by the protocol. The flow cell was then washed with
15 water to remove cells not retained by the positive bias by pumping buffer in a reverse
direction at a rate of 114 μ l/min for 10 minutes. The fluid content of the flow cell was then
exchanged with a solution of SDA reagents for amplifying either the *spa Q* or *inv A* gene
10 sequences.

20 The total nucleic acid from the bacteria cells was isolated by first lysing the cells
by energizing the electrodes with pulsed direct current at 200 V having a square wave
form of 10 ms duration and a total of 40 separate pulses. (Alternatively, the cells can be
25 lysed by heating the chamber to 95°C for five minutes). A concentrated SDA reagent and
buffer stock mix was introduced into the flow cell and mixed with the denatured target
15 nucleic acid to give the following final concentrations of SDA reaction components: 500
nM amplification primers, 50 nM 'bumper' primers, 9.5 mM magnesium acetate, 35 mM
potassium phosphate buffer pH 7.6, 80 μ g/ml bovine serum albumin, and 1.4 mM each of
30 dATP, dGTP, TTP, and alpha-thiolated dCTP. The amplification primers were designed to
20 amplify 81 base pairs of the *invA* or *spa Q* gene and comprised the following nucleic acid
sequences:

35 *spa Q* Seq. Id. No. 1 5'-accgcatcgaatgcattctcgggtcctggtagggttattc-3'

spa Q Seq. Id. No. 2 5'-cgattccgctccagacttctcgggaacacacgccaagta-3'

inv A Seq. Id. No. 3 5'-accgcatcgaatgcattctcgggttcaacgtttcctcgcg-3'

40 *inv A* Seq. Id. No. 4 5'-cgattccgctccagacttctcgggatcgataatgccagacg-3'

25 Bumper primers comprised the following sequences:

spa Q Seq. Id. No. 5 5'-gcaacgattatcggc-3'

45 *spa Q* Seq. Id. No. 6 5'-ccagacagtaaaaac-3'

inv A Seq. Id. No. 7 5'-ttgacagaatcctca-3'

30 *inv A* Seq. Id. No. 8 5'-taagacggctggta-3'

The released nucleic acid was then denatured by heating the chamber to 90°C for five minutes. The chamber was then brought to 60°C and 10 µl of SDA reagent buffer containing enzymes was introduced into the chamber to initiate amplification (i.e., 40 units of BsoB1 restriction endonuclease and 16 units of *exo*-Bst DNA polymerase).

In the system of the invention, amplification using SDA may be carried out for between 25 and 35 minutes. In the present example, after 30 minutes of reaction a 5 µl aliquot of the reaction volume was removed for PAGE analysis (Figs. 19 and 20).

The third stage of sample handling comprising detection of the molecules of interest is preferably carried out for proteins and nucleic acids of interest. Such detection can use various forms of hybridization to probes previously attached to the microarray. For example, nucleic acids (e.g., RNA, DNA, and pNA) may be used for binding sample-derived nucleic acid analyte (e.g., amplified or unamplified target nucleic acids) by hybridization. Proteins may also be made to bind to capture molecules (i.e., protein-ligand binding interactions) attached to the array. Such capture molecules may comprise proteins or other molecules and the binding interaction can comprise such interactions as antigen-antibody, enzyme-substrate, and receptor-ligand binding.

With respect to nucleic acids, target species, whether amplified or not, are electronically addressed to specified capture pads of the microarray of either the single (or the secondary) flow chamber for capture by oligonucleotide probes that are anchored thereto. Preferably, the electrode array of the flow cell, (i.e., flow cell 11, 31a or 32 depending upon the embodiment and protocol used) has at least 25 individually-addressable electrodes coated with a permeation layer (e.g. for example, an acrylamide-based hydro-gel). The target nucleic acid is biased using a positive sinusoidal signal generated using a function generator/arbitrary wave form generator (33120A, Hewlett Packard, Santa Clara, CA). The capture probe-target hybrids are then detected using fluorophore-labeled reporter probes and the CCD-based optical imaging system employed for the portable instrument shown in Figure 2. Detection with this arrangement takes approximately 5 min to accomplish.

Continuing with the above example, the amplification products of the *spa Q* and *inv A* genes were available for capture and analysis. Prior to capture, the SDA reaction

5 solution and amplification products were passed through a desalting column in a volume
of about 75 μ l followed by exchanging the buffer with a 50 mM Histidine buffer. The
10 amplification products were then addressed to specific pads of the electrodes which
contained gene specific probes attached to the permeation layer overlaying the electrodes.
5 This was followed by washing the chamber with 200mM NaCl, 10 mM Tris pH 8.0, 1
mMEDTA, followed in turn by introduction of a bodipy 630-labeled probe
15 oligonucleotide specific for the amplification product at a concentration of 0.5 μ M in 200
mM NaCl, 10 mM Tris pH 8.0, 1 mM EDTA, and 100 μ g/ml Calf Thymus DNA. The
reporter probe solution was left in the assay cell for 10 minutes then washed with 7 – 800
20 μ l of 50 mM Histidine buffer. The chamber was then visualized using a 630 nm helium-
neon laser and a computer controlled CCD camera. Figure 21 shows results of binding of
the *spa Q* gene amplification product to individual pads of the electrode in the flow
chamber. The binding shows that the amplification product produced in the flow cell
25 annealed to the capture pads (fraction 1 and 2) satisfactorily in comparison to annealing of
amplification product produced as a control in a reaction tube and then introduced into the
flow cell chamber after the fractions 1 and 2 had been addressed. The level of binding for
the control amplified product is higher due to the higher level of amplification obtained in
30 the control reaction. We note that very little nonspecific binding of amplified product
from the flow cell reaction bound to a capture site having a nonspecific capture probe.

20 In Figure 22, the amplified SDA products for the *spa Q* gene were also addressed
to specific pads on the microarray using two alternate biasing formats. In one biasing
35 protocol, the amplicons were addressed using a sinusoidally based AC format of 3.5 V
peak-to-peak at 1 kHz. This protocol also used a DC offset voltage of +2.3 V for 5
minutes. In the other protocol, a direct current (DC) format was used comprising +2.5 V
40 25 for 2 minutes. As can be seen, the DC protocol is able to transport the molecules of
interest to the capture sites with greater efficiency as the DC biased pads exhibit a greater
binding signal. This result further shows the versatility of the system in that processing of
sample materials (cells and molecules) may be manipulated in a wide range of adjustments
45 that is possible using this electronic-based lab-on-a-chip system. For example, the use of
30 an AC format though shown in the above example as providing less transport mobility to
the specific sequence, can be performed where the voltage range is between 2.0 and 5.0

5 peak-to-peak, the frequency range is between 1 and 10 kHz, and the DC offset is in a range of 1-5 volts). This versatility of ranges provides for transport of molecules under variable buffer conditions.

10 Visual detection of amplification reaction products by probe hybridization may be performed by directing a battery operated diode laser having at least the capability of generating approximately 2 mWatts of laser power with an emission wavelength of 635 nm. The laser is used to excite fluorescent dye-label reporter probe (such as BODIPY-15 630). The wavelength of the emission filter is 670 nm. The dichromatic mirror has a wavelength cutoff at 645nm. Alternatively, a direct electrochemical voltammetric detection system may also be used instead of light based detection as is understood by 10 those skilled in the art.

20 Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be readily 25 apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

What is claimed is:

1. An integrated system for the analysis of eukaryotic and/or prokaryotic cells in a biological sample comprising:

a. an electronic system for cell separation, cell lysis, sample preparation, and sample analysis further comprising:

at least one input and at least one output port connected to a flow cell, a plurality of individually addressable electrodes positioned within said flow cell, and a heating element connected to said flow cell, said flow cell adapted to receive said sample via the input, said electrodes being operatively coupled to a power source adapted to electronically disrupt cell membranes within said sample, said electrodes further comprising an active matrix array adapted to bind to predetermined molecules of interest within said sample for analysis of said molecules;

b. a detector operatively positioned to detect said molecules bound to said active matrix array by a detectable signal; and

c. a power source.

2. An integrated system of claim 1 wherein the sample preparation further includes the step of cell separation.

3. An integrated system of claim 2 wherein the cell separation is performed electronically.

4. An integrated system of claim 1 further comprising an illumination source operatively positioned to provide radiation to the active matrix array.

5. An integrated system of claim 4 wherein the illumination source is a laser.

6. An integrated system of claim 5 wherein the laser is a diode laser.

7. An integrated system of claim 5 wherein the system includes a beam splitter.

5 8. An integrated system of claim 7 wherein the beam splitter is positioned to receive the illumination and reflect at least a portion thereof to the active matrix array, and to pass at least a portion of the radiation from the active matrix array.

10 9. An integrated system of claim 1 wherein said plurality of individually addressable electrodes comprising the active matrix array are used as a unit for each of said cell separation, cell lysis, and sample analysis.

15 10. An integrated system of claim 1 wherein said plurality of individually addressable electrodes comprising the active matrix array are used independent of one another in the formation of subsets of electrodes for cell separation, cell lysis, and sample analysis.

20 11. An integrated system of claim 1 wherein the sample preparation includes further reaction steps after said electronic lysis.

25 12. An integrated system of claim 11 wherein the further reaction includes enzymatic reaction.

30 13. An integrated system of claim 11 wherein the further reaction includes chemical reaction.

35 14. An integrated system of claim 11 wherein the further reaction includes protease reactions.

40 15. An integrated system of claim 14 wherein the protease reaction includes protease-K reaction.

45 16. An integrated system of claim 1 wherein the further reaction includes an amplification reaction.

50 17. An integrated system of claim 16 wherein the amplification includes SDA.

5

18. An integrated system of claim 16 wherein the amplification includes PCR.

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19. An integrated system of claim 16 wherein the amplification includes linear
5 amplification.

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20. An integrated system of claim 16 wherein the amplification includes
exponential amplification.

10

21. An integrated system of claim 1 wherein the power source provides a time
20 varying signal to at least certain of the electrodes.

20

22. An integrated system of claim 21 wherein the time varying signal includes
an AC component.

25

15

23. An integrated system of claim 22 wherein the AC component is sinusoidal.

30

24. An integrated system of claim 21 wherein the time varying signal includes
an offset signal.

20

25. An integrated system of claim 24 wherein the offset is a DC offset.

35

26. An integrated system of claim 1 further including a control system.

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25 27. An integrated system of claim 26 wherein the control system is a computer.

28. An integrated system of claim 27 wherein the computer is a personal
computer.

45

30 29. An integrated system of claim 28 wherein the personal computer is a
notebook computer.

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55

5 30. An integrated system of claim 1 wherein the flow cell of the electronic system includes at least one port in addition to the input and the output ports.

10 31. An integrated system of claim 30 wherein the flow cell of the electronic
5 system includes at least two ports in addition to the input and the output ports.

15 32. An integrated system of claim 31 wherein the ports are at substantially 90 degrees to another port.

20 33. An integrated system of claim 30 wherein at least two of the ports are in
20 opposition to one another.

 34. An integrated system of claim 1 wherein the detector is a CCD detector.

25 35. An integrated system of claim 1 wherein the system is a closed system.

30 36. An integrated system of claim 1 wherein the system is a self contained system.

35 37. An integrated system of claim 1 wherein the system further includes a fluidic system.

 38. An integrated system of claim 37 wherein the fluidic system includes a
40 pump.

45 39. An integrated system of claim 37 wherein the fluidic system includes a
25 plurality of valves.

50 40. An integrated system of claim 1 wherein the sample analysis includes
30 binding of molecules to the array selected from the group consisting of hybridization and protein-ligand binding interactions.

5 41. An integrated system of claim 40 wherein the hybridization is DNA-DNA hybridization.

10 42. An integrated system of claim 40 wherein the hybridization is DNA/RNA
5 hybridization.

15 43. An integrated system of claim 40 wherein the hybridization is pNA/nucleic acid hybridization.

20 44. An integrated system of claim 40 wherein the protein-ligand binding is selected from the group consisting of receptor-ligand, antigen-antibody, and enzyme-substrate binding.

25 45. An integrated system for the analysis of a biological sample containing eukaryotic and/or prokaryotic cells, comprising:

15 a. a first electronic system for sample preparation, said system further comprising:

30 at least one input port, a first flow cell connected to the input, a plurality of electrodes positioned within said flow cell, and at least one output port, said flow cell adapted to receive said sample via the input, said electrodes being
20 operatively coupled to a power source adapted to electronically disrupt cell membranes within said sample;

35 b. a second electronic system for sample analysis, said system further comprising:

40 at least one input port, a second flow cell connected to the input, a plurality of electrodes positioned within said second flow cell, said electrodes comprising an active matrix array
45 adapted to bind to predetermined molecules of interest within said sample for analysis of said molecules, and at
30 least one output port, said input of the second electronic

5 system being coupled to at least one output port of the first electronic system;

- 10 c. a detector operatively positioned to detect said molecules bound to said active matrix array by a detectable signal; and
5 d. a power source.

15 46. An integrated system of claim 45 wherein the sample preparation further includes the step of cell separation.

10 47. An integrated system of claim 46 wherein the cell separation is performed electronically.
20

25 48. An integrated system of claim 45 further comprising an illumination source operatively positioned to provide radiation to the active matrix array.
15

49. An integrated system of claim 48 wherein the illumination source is a laser.

30 50. An integrated system of claim 49 wherein the laser is a diode laser.

20 51. An integrated system of claim 48 wherein the system includes a beam splitter.
35

40 52. An integrated system of claim 51 wherein the beam splitter is positioned to receive the illumination and reflect at least a portion thereof to the active matrix array, and to pass at least a portion of the radiation from the active matrix array.
25

45 53. An integrated system of claim 45 wherein said plurality of individually addressable electrodes of said first electronic system are used as a unit for each of said cell separation and cell lysis.
30

50 54. An integrated system of claim 45 wherein said plurality of individually addressable electrodes of said second electronic system are used as a unit for said analysis.
55

5

10

55. An integrated system of claim 45 wherein said plurality of individually addressable electrodes of said first electronic system are used independent of one another in the formation of subsets of electrodes for cell separation and cell lysis.

5

15

56. An integrated system of claim 45 wherein said plurality of individually addressable electrodes of said second electronic system are used independent of one another in the formation of subsets of electrodes for said analysis.

10

20

57. An integrated system of claim 45 wherein the sample preparation includes further reaction steps after said electronic lysis.

25

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58. An integrated system of claim 57 wherein the further reaction includes enzymatic reaction.

30

59. An integrated system of claim 57 wherein the further reaction includes chemical reaction.

20

60. An integrated system of claim 57 wherein the further reaction includes protease reactions.

35

61. An integrated system of claim 60 wherein the protease reaction includes protease-K reaction.

40

25

62. An integrated system of claim 57 wherein the further reaction includes an amplification reaction.

45

63. An integrated system of claim 62 wherein the amplification includes SDA.

30

64. An integrated system of claim 62 wherein the amplification includes PCR.

50

55

5 65. An integrated system of claim 62 wherein the amplification includes linear amplification.

10 66. An integrated system of claim 62 wherein the amplification includes
5 exponential amplification.

15 67. An integrated system of claim 45 wherein the power source provides a time varying signal to at least certain of the electrodes.

20 68. An integrated system of claim 67 wherein the time varying signal includes
10 an AC component.

 69. An integrated system of claim 68 wherein the AC component is sinusoidal.

25 70. An integrated system of claim 67 wherein the time varying signal includes
15 an offset signal.

30 71. An integrated system of claim 70 wherein the offset is a DC offset.

20 72. An integrated system of claim 45 further including a control system.

35 73. An integrated system of claim 72 wherein the control system is a computer.

40 74. An integrated system of claim 73 wherein the computer is a personal
25 computer.

45 75. An integrated system of claim 74 wherein the personal computer is a notebook computer.

50 76. An integrated system of claim 45 wherein the first flow cell of the first
30 electronic system includes at least one port in addition to the input and the output ports.

5 77. An integrated system of claim 76 wherein the first flow cell of the first electronic system includes at least two ports in addition to the input and the output ports.

10 78. An integrated system of claim 77 wherein the ports are at substantially 90
5 degrees to another port.

15 79. An integrated system of claim 76 wherein at least two of the ports are in opposition to one another.

20 80. An integrated system of claim 45 further including at least one heater element.

25 81. An integrated system of claim 80 wherein a heater is disposed on the first electronic system.

30 82. An integrated system of claim 80 wherein a heater is disposed on the second electronic system.

35 83. An integrated system of claim 80 wherein the heater element is in thermal
20 contact with either of the first or second electronic systems.

40 84. An integrated system of claim 45 wherein the detector is a CCD detector.

45 85. An integrated system of claim 45 wherein the system is a closed system.

50 86. An integrated system of claim 45 wherein the system is a self contained system.

55 87. An integrated system of claim 45 wherein the system further includes a fluidic system.

5 88. An integrated system of claim 87 wherein the fluidic system includes a pump.

10 89. An integrated system of claim 87 wherein the fluidic system includes a
5 plurality of valves.

15 90. An integrated system of claim 45 wherein the sample analysis includes binding of molecules to the array selected from the group consisting of hybridization and protein-ligand binding interactions.

20 91. An integrated system of claim 90 wherein the hybridization is DNA-DNA hybridization.

25 92. An integrated system of claim 90 wherein the hybridization is DNA/RNA
15 hybridization.

30 93. An integrated system of claim 90 wherein the hybridization is pNA/nucleic acid hybridization.

35 94. An integrated of claim 90 wherein the protein-ligand binding is selected from the group consisting of receptor-ligand, antigen-antibody, and enzyme-substrate binding.

40 95. An integrated system for the analysis of a biological sample containing eukaryotic and/or prokaryotic cells, comprising:

45 a. a first nonelectronic system for sample preparation, said system further comprising:

50 at least one input port, a first flow cell connected to the
45 input, a heater element, and at least one output port, said
30 flow cell adapted to receive said sample via the input, said
heater being operatively coupled to a power source adapted

5 to heat said flow cell to a temperature sufficient to induce
disruption of cell membranes within said sample;

10 b. a second electronic system for sample analysis, said system further
comprising:

5 at least one input port, a second flow cell connected to the
input, a plurality of electrodes positioned within said second
15 flow cell, said electrodes comprising an active matrix array
adapted to bind to predetermined molecules of interest
within said sample for analysis of said molecules, and at
10 least one output port, said input of the second electronic
20 system being coupled to at least one output port of the first
nonelectronic system;

25 c. a detector operatively positioned to detect said molecules bound to
said active matrix array by a detectable signal; and

15 d. a power source.

30 96. An integrated system of claim 95 further comprising an illumination source
operatively positioned to provide radiation to the active matrix array.

20 97. An integrated system of claim 96 wherein the illumination source is a laser.

35 98. An integrated system of claim 97 wherein the laser is a diode laser.

40 99. An integrated system of claim 96 wherein the system includes a beam
25 splitter.

45 100. An integrated system of claim 99 wherein the beam splitter is positioned to
receive the illumination and reflect at least a portion thereof to the active matrix array, and
to pass at least a portion of the radiation from the active matrix array.

30 101. An integrated system of claim 95 wherein said plurality of individually
50 addressable electrodes of said electronic system are used as a unit for said analysis.

5 102. An integrated system of claim 95 wherein said plurality of individually addressable electrodes of said electronic system are used independent of one another in the formation of subsets of electrodes for said analysis.

10 103. An integrated system of claim 95 wherein the sample preparation includes further reaction steps after said heater induced lysis.

15 104. An integrated system of claim 103 wherein the further reaction includes enzymatic reaction.

20 105. An integrated system of claim 103 wherein the further reaction includes chemical reaction.

25 106. An integrated system of claim 103 wherein the further reaction includes protease reactions.

30 107. An integrated system of claim 106 wherein the protease reaction includes protease-K reaction.

35 108. An integrated system of claim 103 wherein the further reaction includes an amplification reaction.

 109. An integrated system of claim 108 wherein the amplification includes SDA.

40 110. An integrated system of claim 108 wherein the amplification includes PCR.

45 111. An integrated system of claim 108 wherein the amplification includes linear amplification.

50 112. An integrated system of claim 108 wherein the amplification includes exponential amplification.

5 113. An integrated system of claim 95 wherein the power source provides a time varying signal to at least certain of the electrodes.

10 114. An integrated system of claim 113 wherein the time varying signal includes
5 an AC component.

15 115. An integrated system of claim 114 wherein the AC component is sinusoidal.

10 116. An integrated system of claim 113 wherein the time varying signal includes
20 an offset signal.

 117. An integrated system of claim 116 wherein the offset is a DC offset.

25 118. An integrated system of claim 95 further including a control system.

30 119. An integrated system of claim 118 wherein the control system is a computer.

20 120. An integrated system of claim 119 wherein the computer is a personal computer.

35 121. An integrated system of claim 120 wherein the personal computer is a notebook computer.

40 122. An integrated system of claim 95 wherein the first flow cell of the nonelectronic system includes at least one port in addition to the input and the output ports.

45 123. An integrated system of claim 122 wherein the first flow cell of the nonelectronic system includes at least two ports in addition to the input and the output ports.
50
55

5

124. An integrated system of claim 123 wherein the ports are at substantially 90 degrees to another port.

10

5 125. An integrated system of claim 122 wherein at least two of the ports are in opposition to one another.

15

126. An integrated system of claim 95 further including at least two heater elements.

10

20

127. An integrated system of claim 126 wherein a heater is disposed on the electronic system.

25

128. An integrated system of claim 126 wherein a heater element is in thermal contact with either of the nonelectronic or electronic systems.

30

129. An integrated system of claim 95 wherein the detector is a CCD detector.

20

130. An integrated system of claim 95 wherein the system is a closed system.

35

131. An integrated system of claim 95 wherein the system is a self contained system.

40

25 132. An integrated system of claim 95 wherein the system further includes a fluidic system.

45

133. An integrated system of claim 132 wherein the fluidic system includes a pump.

30

134. An integrated system of claim 132 wherein the fluidic system includes a plurality of valves.

50

55

5 135. An integrated system of claim 95 wherein the sample analysis includes
binding of molecules to the array selected from the group consisting of hybridization and
protein-ligand binding interactions.

10 5 136. An integrated system of claim 135 wherein the hybridization is DNA-DNA
hybridization.

15 137. An integrated system of claim 135 wherein the hybridization is DNA/RNA
hybridization.

10 20 138. An integrated system of claim 135 wherein the hybridization is
pNA/nucleic acid hybridization.

25 15 139. An integrated of claim 135 wherein the protein-ligand binding is selected
from the group consisting of receptor-ligand, antigen-antibody, and enzyme-substrate
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1/22

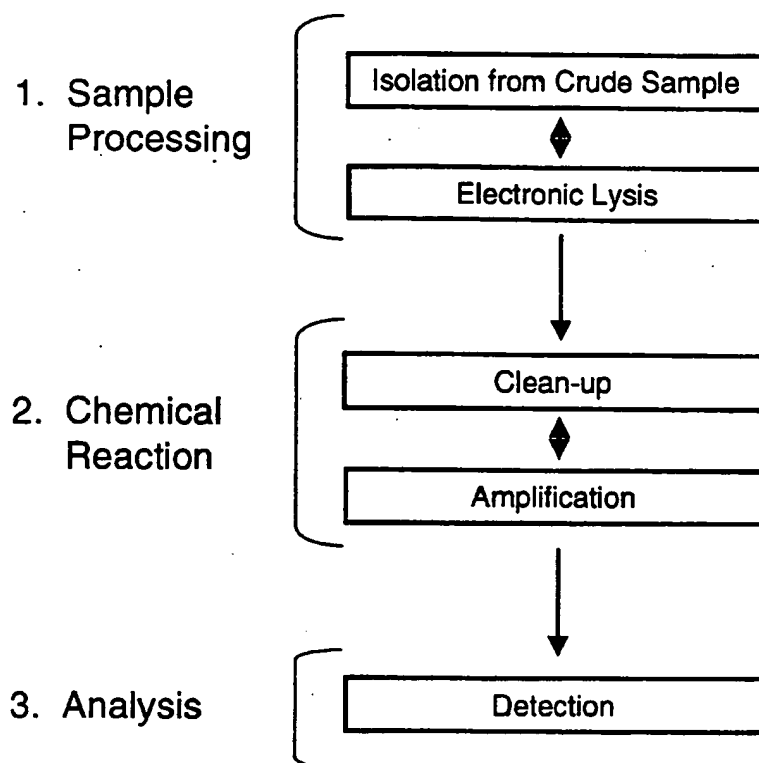


Fig. 1

2/22

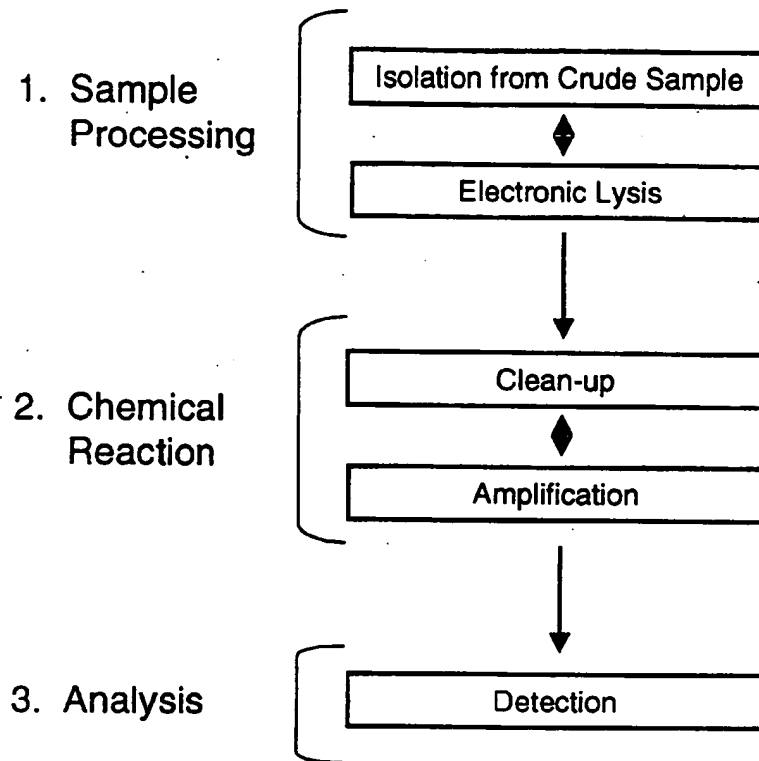


Fig. 1

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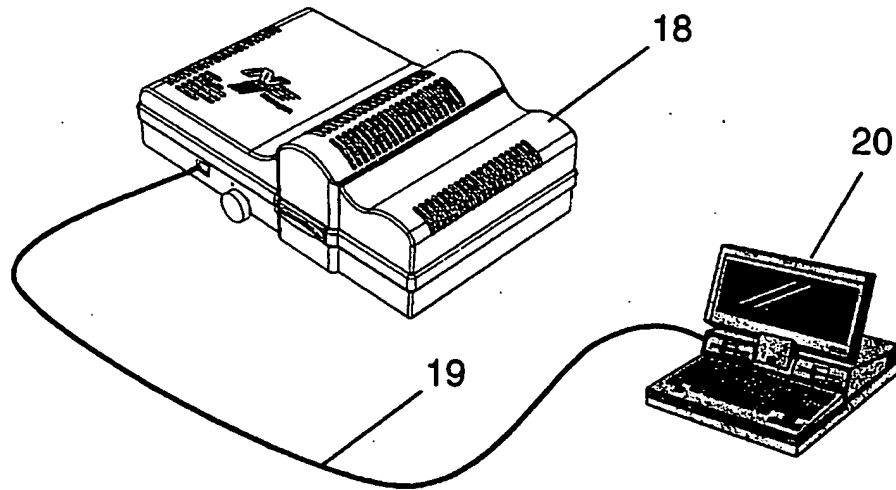


Fig. 3

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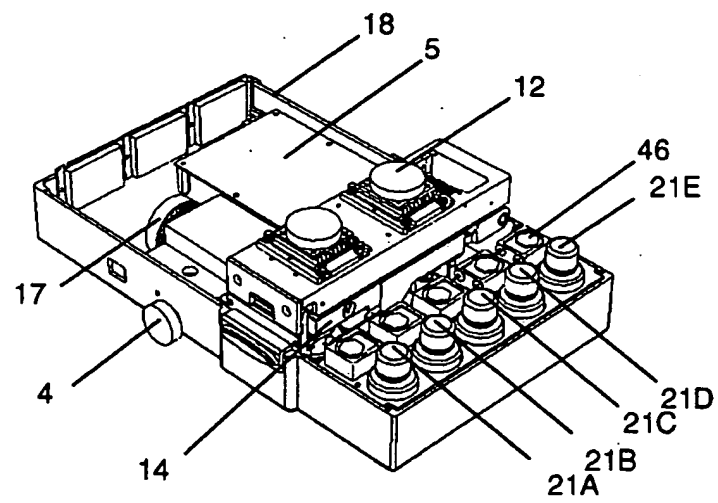


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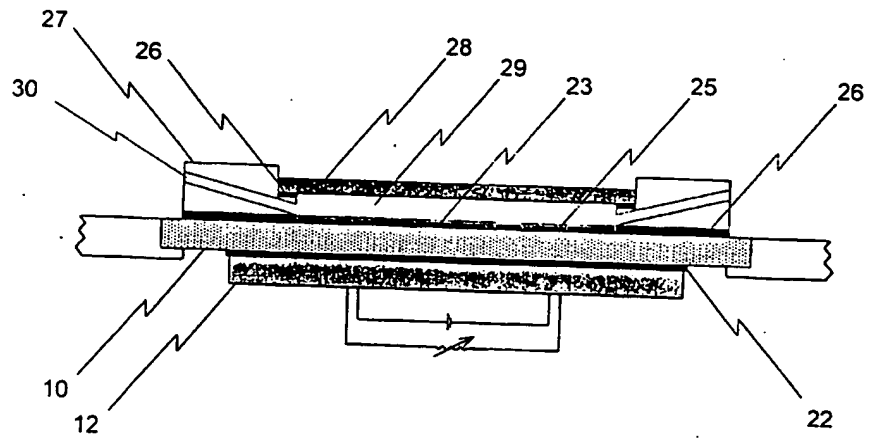


Fig. 5

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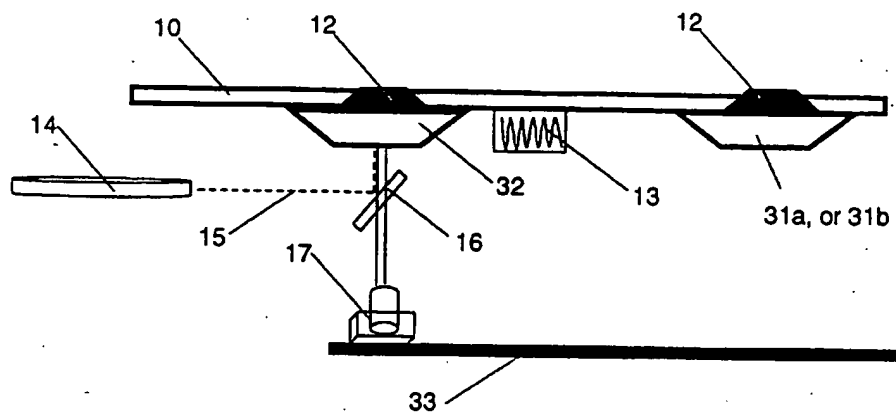


Fig. 6

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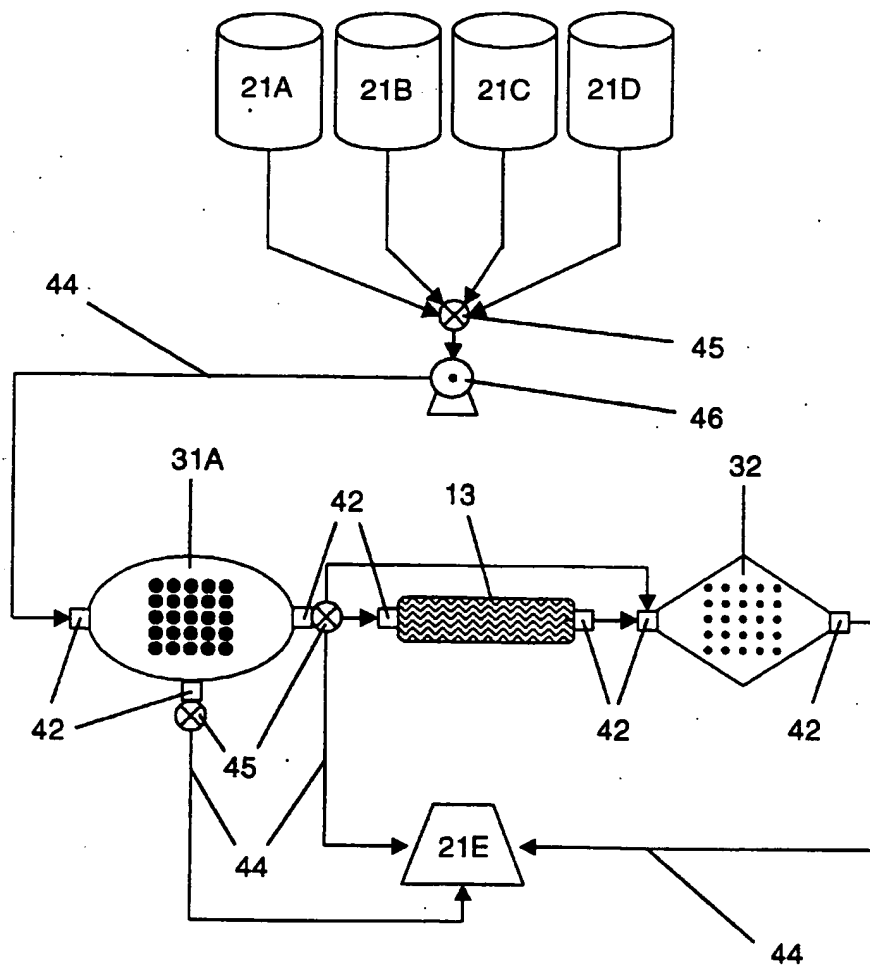


Fig. 7

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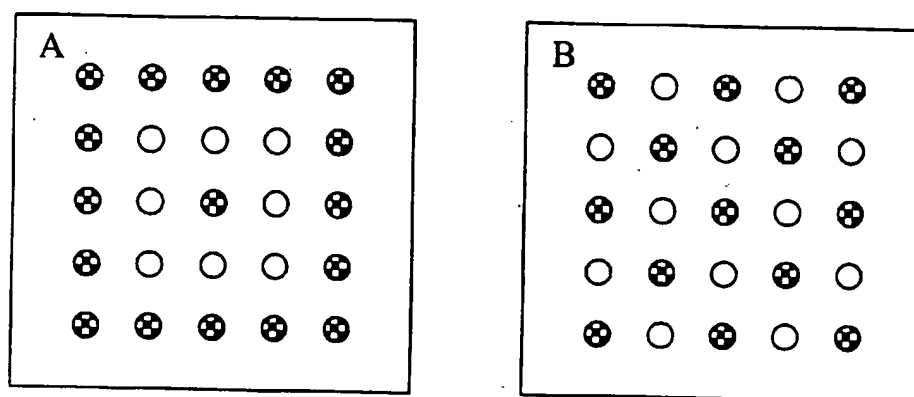


Fig. 8

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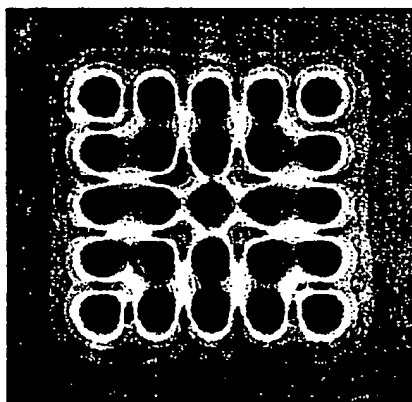


Fig. 9

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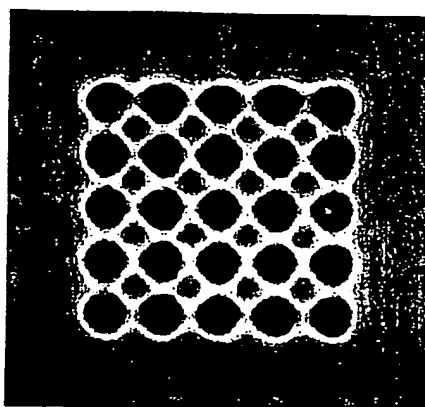


Fig. 10

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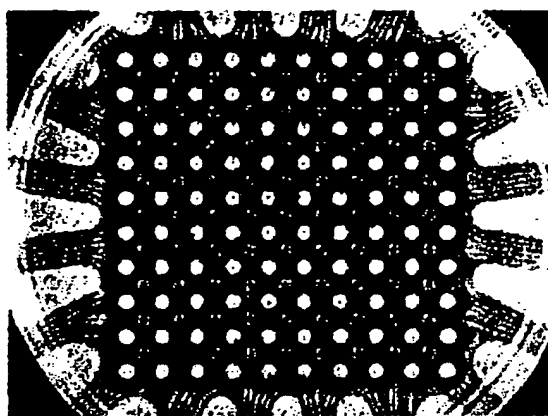


Fig. 11

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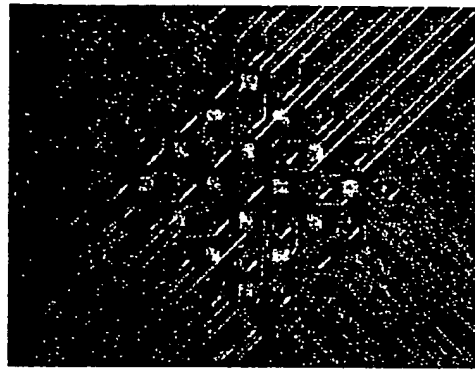


Fig. 12

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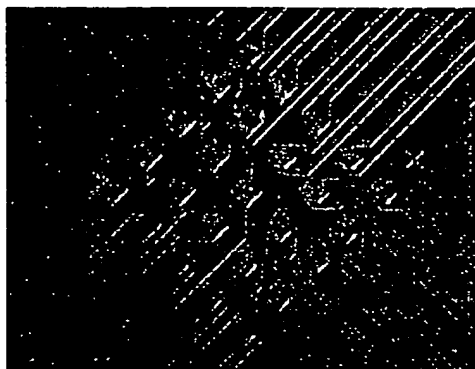


Fig. 13A

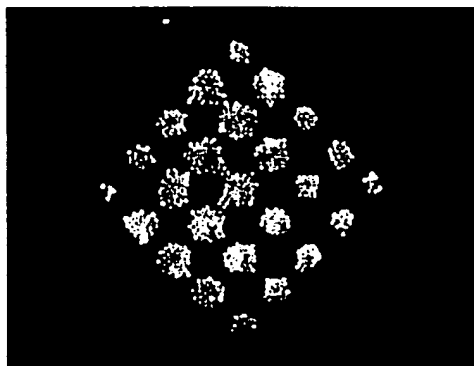


Fig. 13B

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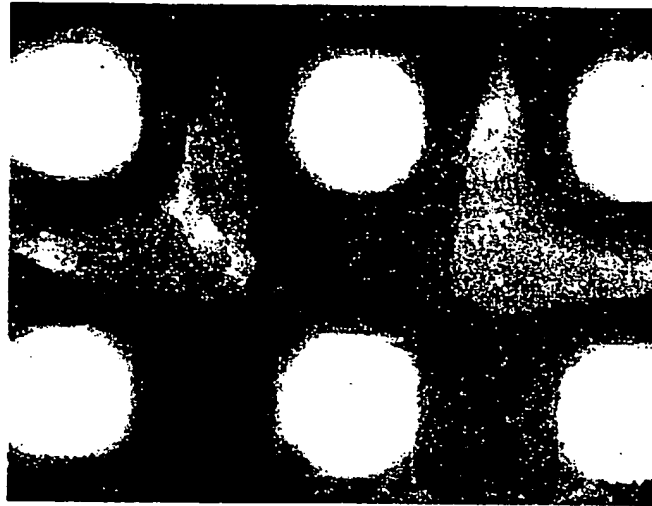


Fig. 14

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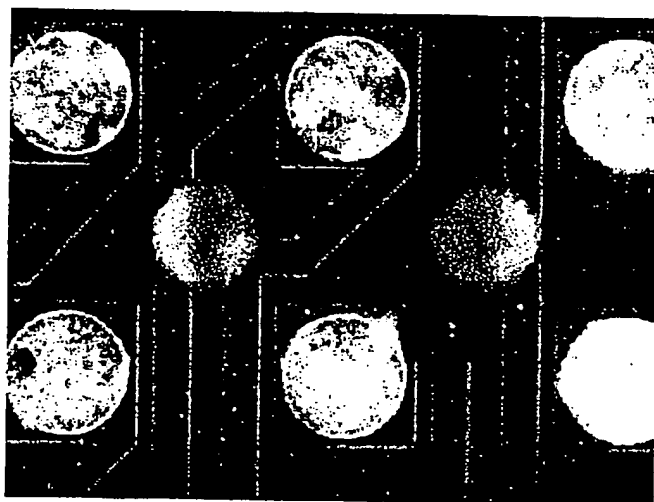


Fig. 15

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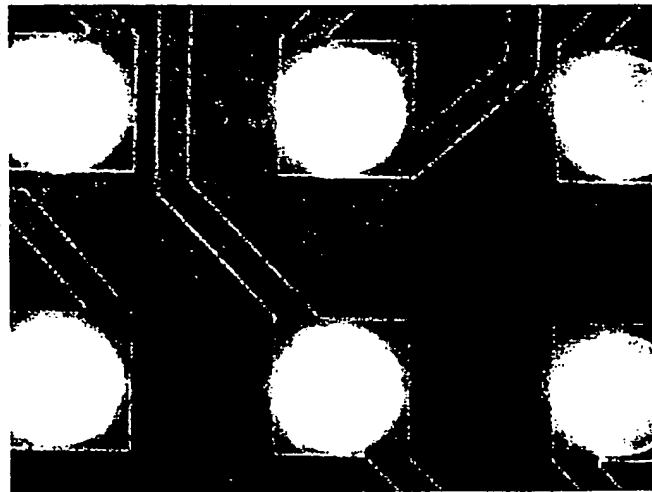


Fig. 16

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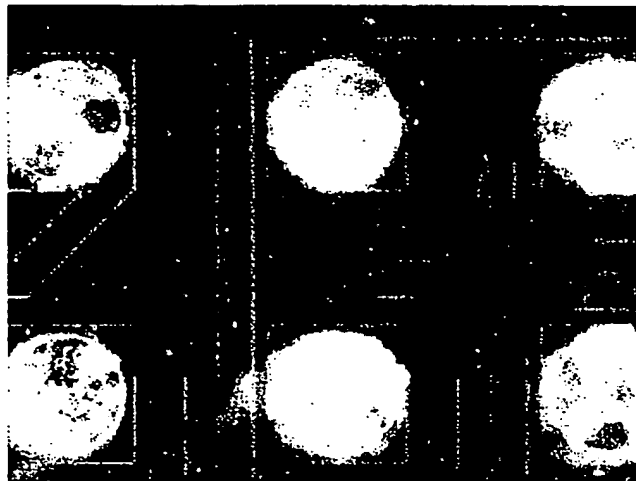


Fig. 17

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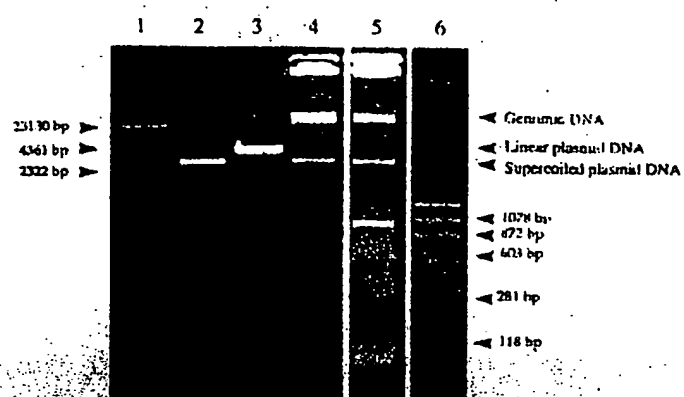


Fig. 18

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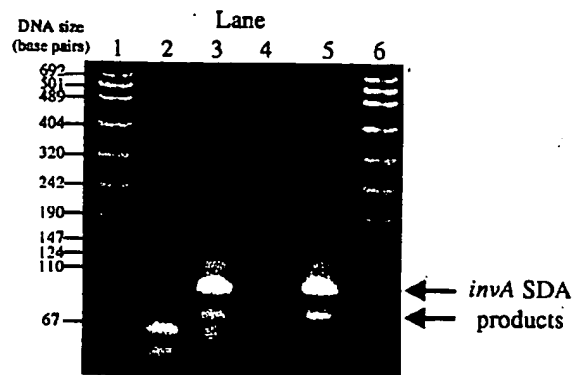


Fig. 19

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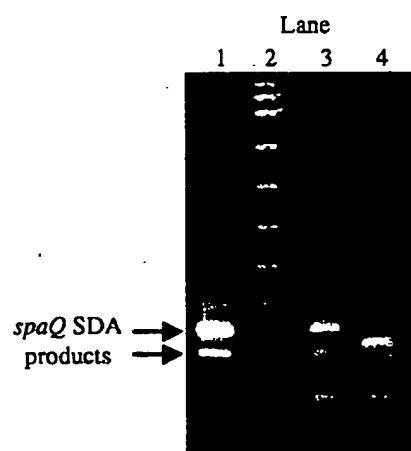


Fig. 20

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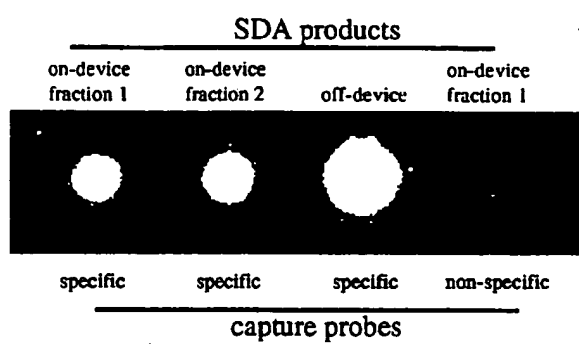


Fig. 21

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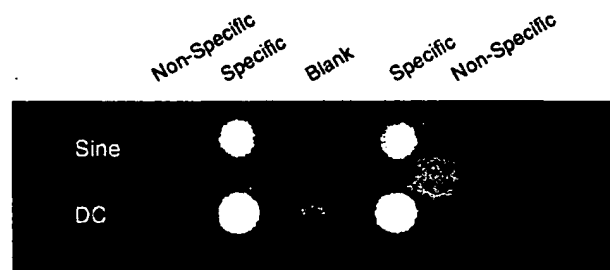


Fig. 22

SEQUENCE LISTING

<110> CHENG, JING
WU, LEI
HELLER, MICHAEL
SHELDON, ED
DIVER, JONATHAN
O'CONNELL, JAMES P.
SMOLKO, DAN
JALALI, SHILA
WILLOUGHBY, DAVID

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<140> To be assigned
<141> Filed herewith

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<151> 1998-01-30

<150> 08/709,358
<151> 1996-09-06

<150> 08/986,065
<151> 1997-12-05

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/31098

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : B01D 57/02; G01N 15/06; C12M 1/36, 1/34

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 204/547, 643; 422/68.1, 81, 82.05; 435/286.5, 287.1, 287.2, 287.3, 288.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,661,451 A (HANSEN) 28 April 1987, see col.2, lines 19- col. 3, lines 42.	1-139
A	US 5,605,662 A (HELLER et al.) 25 February 1997, see entire disclosure.	1-139
A	US 5,795,457 A (PETHIG et al.) 18 August 1998, see description of figure 8.	1-139

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

Special categories of cited documents:	
A document defining the general state of the art which is not considered to be of particular relevance	*T* later documents published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
E earlier document published on or after the international filing date	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
O document referring to an oral disclosure, use, exhibition or other means	*A* document member of the same patent family
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

21 MARCH 2000

Date of mailing of the international search report

04 APR 2000

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Commissioner of Patents and Trademarks
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/31098

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

204/547, 643; 422/68.1, 81, 82.05; 435/286.5, 287.1, 287.2, 287.3, 288.7